

PATENT

Case Docket No.: NOVCEL.3CPDDDVC

Applicant(s)	:	Hubbell, et al.
App. No.	:	10/761,180
Filed	:	January 20, 2004
For	:	Gels For Encapsulation Of Biological Materials
Examiner	:	Berman, Susan W.
Group Art Unit	:	1711



DECLARATION UNDER 37 C.F.R. §1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, AMARPREET S. SAWHNEY, a citizen of the United States, hereby declare that:
 2. My address is 101A First Ave., Waltham, MA 02451.
 3. I am a co-inventor of the invention in U.S. Application Serial No. 10/761,180 entitled “Gels For Encapsulation Of Biological Materials” (the “Application”); and U.S. Application Serial No. 07/843,485 (“Priority Document I”), now abandoned, filed February 28, 1992 and U.S. Application Serial No. 07/870,540 (“Priority Document II”), filed April 20, 1992, now abandoned, on which this application claims priority.
4. I understand that the Examiner has cited Soon-Shiong et al., U.S. Patent Numbers 5,700,848; 5,705,270 and 5,846,530 in her rejection of the claims presented in the Application. I also understand that Soon-Shiong ‘848, ‘270 and ‘530 have effective filing dates of October 29, 1991.
5. Prior to October 29, 1991; I reduced to practice in the United States a method of encapsulating biological materials, which comprises mixing the biological material with a polymer, forming microcapsules, coating the microcapsules with a photoinitiator, mixing the microcapsules containing the biological material in an aqueous macromer, polymerizing the gel using a light source, and generating macrocapsules in accordance with the subject matter claimed

in this application.

6. Attached hereto is a copy of a page from my laboratory notebook ("Exhibit 1"), which shows a protocol for encapsulating cells in alginate-PLL microcapsules. Except for the masked date, Exhibit 1 is a true and correct copy of the relevant page in the original bound laboratory notebook.

7. Attached hereto is a copy of a page from my laboratory notebook ("Exhibit 2") bearing a date before October 29, 1991. Except for the masked date, Exhibit 2 is a true and correct copy of the relevant page in the original bound laboratory notebook. Exhibit 3 is a transcription of the handwriting of Exhibit 2.

8. Exhibit 2 shows the protocol for encapsulating alginate-PLL microcapsules in a photopolymerized PEO gel. The steps in the protocol correspond to the steps in Claim 148 of the application.

9. Accordingly, Exhibit 1 and Exhibit 2 demonstrate that the invention as described in claim 148 in the Application was conceived and reduced to practice in the United States before the filing date of Soon-Shiong '848.

10. I understand that the Examiner has also cited Soon-Shiong et al., U.S. Patent Numbers 5,545,423; 5,759,578; 5,788,988 and 5,879,709 in her rejection of the claims presented in the Application. I understand that Soon-Shiong '423, '578, '988, and '709 have effective filing dates of November 25, 1991, which is later than Soon-Shiong '848. Therefore, as shown above, the invention as described in claim 148 was conceived and reduced to practice in the United States before the effective filing date of Soon-Shiong '423.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date:

9/17/05

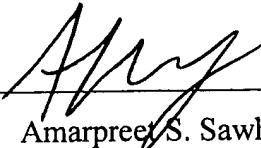

Amarpreet S. Sawhney

EXHIBIT 1

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Fluid setup

Procedure for Microencapsulation

A syringe pump with a 10-ml syringe connected to a special air jet is used for making sodium alginate islet droplets.

For each preparation, 2000–3000 islets in 0.2 ml of saline is mixed gently with 2 ml of 1.5% sodium alginate, transferred to the 10-ml syringe, and connected to the air jet. The distance from the tip of the air jet needle to the surface of the collecting fluid is set at precisely 8 cm. The syringe pump and air flow are then turned on to extrude sodium alginate droplets containing islets into 50 ml of the 1.1% CaCl_2 solution in a beaker. During extrusion, the islets are kept in suspension by gently rotating a small magnet inside the 10-ml syringe. After the extrusion process is complete, the spherical calcium alginate gel droplets are transferred to a 50-ml polystyrene test tube with a conical bottom, and allowed to settle before withdrawing the supernatant down to 5 ml using a vacuum aspirator. The gel droplets are washed once with 30 ml of 0.55% CaCl_2 , once with 0.2% CaCl_2 , and then suspended in 25 ml of 0.1% CHES solution for 3 min. After aspirating the CHES solution, the capsules are washed with 1% CaCl_2 and suspended in 25 ml of 0.05% (W/V) poly(L-lysine) for 6 min. After further washing with CHES, CaCl_2 , and saline, the microcapsules are incubated in 0.15% sodium alginate for 4 min and washed with saline. The capsules are then suspended in 10 ml of 55 mM sodium citrate solution for 5 min. The final product is washed twice with saline and once with medium CMRL-1969, and then transferred to Falcon flasks for incubation at 37° until required for *in vitro* and *in vivo* studies.

distance
should be
8 cm

1.1

1.2

1. Poly(L-lysine) + CHES + add 1mM Ca^{2+}

2. CHES, 3. SALINE

1400 mL | mins on test tube on wall (air-pressure)

EXHIBIT 2

PROTOCOL FOR PLL - MICROSPHERES COATING WI- PEG GELS

Make alginate PLL microspheres & suspend them in a saturated (freshly made) solution of ethyl eosin in a HEPES buffered saline. Aspirate off excess & CaCl_2 solⁿ from spheres & add 10 ml of dye solⁿ. Incubate for 5 min. Rinse off repeatedly (about 4-5 times) w/ 1.1% CaCl_2 .

Aspirate 0.5 ml spheres into 15 ml centrifuge tubes. Aspirate off all excess fluid. Add 100 μl of TGA solⁿ () and add 2 ml of 3.0% PEG acrylate solⁿs. Vortex & expose to laser (514nm) at ~100 mW for ~1-2 min with ~~constant~~ intermittent stirring. Add 10 ml saline to wash off excess polymer, aspirate & resuspend w/ extracellular solⁿ to allow spheres to reswell. Wash twice w/ extracellular solⁿ & then w/ saline. Suspend in 5 ml saline & implant in 2 mice.

EXHIBIT 3

EXHIBIT 2
Transcription of handwriting of Exhibit 1

PROTOCOL FOR PLL-MICROSPHERE COATING W/ PEO GELS

Make algin -/PLL microspheres & immerse them in a saturated (freshly made) solution of ethyl eosin in HEPES buffered saline. Aspirate of¹ all excess CaCl₂ sol² from spheres (5 ml) and add 10 ml of dye sol³. Incubate for 5 min. Rinse off repeatedly (about 4 – 5 times) w/- 1.1% CaCl₂.

Aliquot 0.5 ml spheres into 15 ml centrifuge tubes. Aspirate off all excess fluid. Add 10 µl of TEA³ sol⁴ () and add 2 ml of 30% PEO⁴ acrylate sol⁵'s. Vortex & expose to laser (514 nm) at ~100 mW for ~1-2 min with intermediate stirring. Add 10 ml saline to wash off excess polymer, aspirate & incubate w/- citrate sol⁶ to allow spheres to re-swell. Wash twice w/- citrate and then w/- saline. Suspend in 5 ml saline & implant in 2 mice.

¹ “of” is off

² “sol²” is solution

³ “TEA” is triethanol amine

⁴ “PEO” is poly(ethylene oxide)